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included in this series chlorthalidone had to be discontinued. One refused the drug after a trial of four weeks, because of postural hypotension. A second suffered from dyspnea, palpitation and orthostatic hypotension of a severe degree. Extreme fatigue and tachycardia led to cessation of administration of the drug in the third after 15 days. In the fourth, a single dose of 300 mg. caused all of the symptoms noted in Table II, and another trial 10 days later produced the same effects. Dizziness, asthenia, palpitations, orthostatic hypotension, anorexia, nausea, and eructation were the reasons for discontinuing the drug in the case of the fifth patient, after a 15-day period of administration. A new trial in this patient four weeks later, with a potassium supplement, resulted in severe hypokalemia and digitalis intoxication which necessitated admission to hospital.

SUMMARY AND CONCLUSIONS

Chlorthalidone, a new oral diuretic, is a sulfamylated benzophenone derivative, chemically different from chlorothiazide, but with a common functional group.

It has a duration of action which is longer than that of any previously observed diuretic. Patients can be maintained by administration of the drug on a three-times-per-week schedule, thus reducing the expense and annoyance of taking large numbers of pills.

As an antihypertensive drug it is comparable to the thiazide derivatives. Of interest is the fact that the maximum fall in blood pressure is rarely seen before the third or fourth day of therapy; in other words, after the maximum saluretic effect. This shows that some degree of salt depletion is greatly beneficial to patients with arterial hypertension.

As a saluretic agent, it is 10 to 20 times more potent than chlorothiazide. The saluresis is most marked during the first three days and decreases greatly during the following days. Like all of the thiazides, chlorthalidone produces increased kaliuresis, especially when used at a dosage above 50 mg./day or when used in long-term therapy. In our experience, all patients receiving 200 mg. per day and 30% of patients on long-term therapy who received an average of 75 mg. per day developed hypokalemia which necessitated the administration of large supplements of potassium.

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Serum Ribonuclease in Patients with Malignant Disease

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ALTHOUGH the human body is deceptively constant in its composition, we know that it is in a continuous state of flux. The dynamic equilibrium existing between the anabolism and catabolism of essential constituents within the cell is reflected in the relatively uniform level of certain specific factors and metabolic end-products in the circulating fluids. Therefore, alterations of activity levels of certain enzymes have come to be recognized as an important feature of the clinical syndromes of certain human diseases, for example the serum phosphatase level, and supply information useful in differential diagnosis and prognosis.

It is hoped that with the introduction of rapid, relatively simple techniques for enzyme estimation, the relationship between enzyme levels and various pathologic states will achieve practical diagnostic importance in the very near future.

Studies on ribonuclease have been directed chiefly to its cellular localization, chemistry and biological activity. Our present knowledge of the serum ribonuclease (S-RNase) level in patients with cancer is rather incomplete, and reports are often contradictory. Métais and Mandel[1] and Houck and Berman[2] were unable to show any difference between the level of serum RNase in normal and cancer patients. Migliarese[3] and Levy and Rottino[4] have reported elevated serum RNase values in patients with carcinoma and in Hodgkin's disease. Of particular interest are the findings of Zigman and Allison[5] which seem to correlate tumour growth with an elevation of serum ribonuclease level in rats bearing transplanted Walker-256 tumours.

The results described here are based on studies of serum ribonuclease determined in four groups

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This research project was supported by a grant-in-aid from the National Cancer Institute of Canada. Presented at the American Chemical Society Meeting held in New York, September 1960.

of subjects: (a) apparently healthy persons; (b) patients with untreated cancer; (c) patients undergoing therapy for cancer, and (d) patients in whom the disease had been arrested and no sign of residual disease was noted when re-examined as part of the follow-up program at the Montreal Cancer Institute of the Notre Dame Hospital.

MATERIALS AND METHODS

A commercial preparation of yeast ribonucleic acid (RNA, Schwarz & Co.), purified as described previously,[6] served as substrate. Blood was collected without the use of anticoagulant. Serum was obtained by allowing the blood to clot for one hour at room temperature and centrifuged for 20 minutes at a temperature of 2-3° C. Serum was diluted with 0.25 M sucrose, making a 10% solution which was used for determination of RNase activity.

RNase activity was determined by the spectrophotometric method based on the measurement of optical density at 260 mμ. of the acid-soluble products formed by the action of RNase. The reaction mixture containing 2.90-2.95 ml. of 0.1 M phosphate buffer pH 7.4, 1.0 ml. of 1% sodium salt of RNA and 0.1-0.05 ml. of 10% solution of serum was incubated for 30 minutes at 37° C. The reaction was stopped by adding 1.0 ml. of 12% solution of $HClO_4$. The mixture was then centrifuged and the absorption of the supernatant, diluted 1:20, was determined at 260 mμ. The control mixtures were incubated in a similar manner except that the serum solutions were added to the precipitated RNA just before centrifugation.

The RNase level was expressed in arbitrary units.* All experiments were carried out in duplicate to confirm that the quantity of products formed was proportional to the quantity of enzyme used.

RESULTS

The S-RNase level of 50 apparently healthy persons was determined and used for comparison. The mean value for this group (with its standard deviation) was calculated to be 16 ± 2.5. Highest and lowest values varied within a range of 11 to 22 units.

Table I summarizes the S-RNase level in 18 cancer patients before therapy. The mean value (30 ± 9.7) was significantly higher than in apparently healthy individuals (16 ± 2.5). The S-RNase level was studied in 22 patients with cancer of various organs who received treatment and in whom the disease was considered to be arrested. Table II shows that the calculated mean value (31 ± 8.4) in this group of patients was found to be outside the normal range. It is apparent also that the choice of therapy had no significant effect on the high S-RNase level registered in this group. The values reported are individual values for each patient under investigation. In seven treated patients, borderline values (less than 24 units) were noted. A persistent elevated S-RNase level is even more evident in a group of patients who had undergone treatment but in whom residual disease was still present, or in whom a recurrence of the tumour was evident. The majority of patients in this group received protracted treatment on repeated occasions and were considered unsuccessfully treated. A significantly high serum ribonuclease level was noted in 18 out of 22 patients (80%) belonging to this group. There is insufficient evidence to correlate a high S-RNase level with evolution or progress of the disease state. Nevertheless, there are some

*One unit is the quantity of serum which when suspended in a millilitre of diluted supernatant gives an optical density of 2.

TABLE I.—SERUM RIBONUCLEASE LEVEL IN 18 UNTREATED CANCER PATIENTS
(mean value 30 ± 9.7)

Patient No.	*Diagnosis*	*Specific activity**
1	Carcinoma of the uterus	40
2	" "	38
3	" "	37
4	" "	33
5	" "	28
6	" "	26
7	" "	24
8	" "	23
9	" "	22
10	" "	20
11	Carcinoma of the breast	24
12	Astrocytoma of the rectum	100
13	Carcinoma of the bronchus and skin	59
14	Carcinoma of the rectum	27
15	Lymphoma of Brill-Symmers type	27
16	Carcinoma of the lip	26
17	Carcinoma of the submaxillary gland	23
18	Carcinoma of the bronchus	22
	Mean	30 ± 9.7†

*For definition of units, see text.
†S-RNase value of patient No. 12 was excluded from the mean.

TABLE II.—SERUM RIBONUCLEASE LEVEL IN 22 TREATED CANCER PATIENTS
(mean value 31 ± 8.4)

Patient No.	*Diagnosis*	*Specific* activity*	*Treatment*
19	Carcinoma of the uterus	38	Radiotherapy
20	" "	37	"
21	" "	26	Cobalt therapy
22	Carcinoma of the breast	42	Surgery
23	" "	36	Radiotherapy
24	" "	35	"
25	" "	31	"
26	" "	27	"
27	" "	27	Surgery
28	" "	26	"
29	Carcinoma of the prostate	50	Radiotherapy
30	Liposarcoma	42	Surgery
31	Buccal carcinoma	37	Radiotherapy
32	Carcinoma of the nasopharynx	34	Surgery, radiotherapy
33	Hodgkin's disease	32	Radiotherapy
34	Carcinoma of the lung	28	"
35	Carcinoma of the skin	27	Surgery
36	" "	27	"
37	Carcinoma of the lip	24	Surgery, radiotherapy
38	Carcinoid of the bronchus	20	Surgery
39	Fibroblastic sarcoma	17	Radiotherapy
40	Carcinoma of the thyroid	15	Surgery
	Mean	31 ± 8.4	

*For definition of units, see text.

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TABLE III.—VARIATIONS IN SERUM RIBONUCLEASE LEVEL IN 9 TREATED CANCER PATIENTS WHO WERE APPARENTLY WITHOUT RESIDUAL DISEASE

Patient No.	*Diagnosis*	*Specific activity**			*Treatment*
		Before treatment	*During treatment*	*After treatment*	
1	Carcinoma of the uterus	40	39	—	Cobalt therapy
2	" "	38	36	27	Radiotherapy
20	" "	37	26	18-27	"
3	" "	37	42	—	"
4	" "	33	—	20	Surgery
6	" "	26	—	22	"
13	Carcinoma of the bronchus	59	44	—	Radiotherapy
14	Carcinoma of the rectum	30	—	46	"
16	Carcinoma of the lip	26	35	—	"

*For definition of units, see text.

indications that such a relationship may exist. This is illustrated by patients Nos. 1, 2, 3, 13, 14, 19 and 20, who showed a high S-RNase level at a later stage of the disease. The elevated serum ribonuclease level noted in a high percentage of patients with recurrence of cancer may therefore be related to evolution and progress of the disease state.

Finally, there is evidence indicating that a lowering of the S-RNase level occurs in patients undergoing treatment when a response to therapy, at least temporary, is achieved in them. This is shown in Table III. In five patients the S-RNase level was determined after treatment was concluded. In four of the five the serum ribonuclease level was lower when the disease was considered to be arrested. This is well illustrated by patient No. 20 who showed a drop in the serum ribonuclease level from 37 to 18 units after protracted deep x-ray therapy for a carcinoma of the uterus. However, a few weeks later, the patient was readmitted to hospital for a recurrence of the disease. On readmission, the S-RNase level had risen to 27 units.

It is apparent, therefore, that in patients in whom no lowering of S-RNase level occurred during or following treatment, the disease could presumably be considered unsuccessfully arrested. This was also noted in patients who were found to have a very high serum ribonuclease level only a few days previous to death.

A group of 36 patients previously treated for cancer, and in whom the disease was considered arrested or in whom there were no signs of residual disease, were reviewed as part of the follow-up program of the Montreal Cancer Institute. The serum ribonuclease level in these cases was determined, and the results are indicated in Table IV. In this group there is a significantly higher S-RNase level in 30% of the patients. The remaining 70% show normal or slightly higher than normal values. No patients in this group had evidence of recurrence of the disease. On the other hand, in one patient (No. 76) with a pigmented melanoma, in whom the tumour appeared well localized, the S-RNase level was found to be within the normal range (17 units).

DISCUSSION

A possible explanation for a high S-RNase level in patients with cancer would be that the enzyme is being released into the blood stream by the involved tissue itself. This would account also for the marked increase in the S-RNase level in patients with more advanced disease when compared with those with an earlier stage of the malignant process.

A high serum RNase level has been noted in patients with cirrhosis of the liver, with leukemia, and in those subjected to trauma.[3, 4] Apparently in these disease states and in trauma there is a release of RNase by the injured tissue.

A persistently high serum ribonuclease level in patients who appear to have been successfully treated may indicate that residual disease is still present and that a latent pathological state exists

TABLE IV.—SERUM RIBONUCLEASE LEVEL OF 36 CANCER PATIENTS R VIEWED IN A FOLLOW-UP PROGRAM

Patient No.	*Diagnosis*	*Specific activity**	*Remarks*
41	Carcinoma of the uterus	30	No sign of residual disease
42	" "	29	" " "
43	" "	29	" " "
44	" "	29	" " "
45	" "	27	" " "
46	" "	24	" " "
47	" "	23	" " "
48	" "	23	" " "
49	" "	22	" " "
50	" "	21	" " "
51	" "	19	" " "
52	Carcinoma of the breast	35	No sign of residual disease
53	" "	32	" " "
54	" "	29	" " "
55	" "	27	Pain in the dorsal region (no treatment)
56	" "	25	No sign of residual disease
57	" "	23	Lymph nodes present—no treatment
58	" "	22	No sign of residual disease
59	" "	22	" " "
60	" "	20	" " "
61	" "	20	" " "
62	" "	19	" " "
63	" "	19	" " "
64	" "	19	" " "
65	" "	18	" " "
66	Carcinoma of the larynx	27	" " "
67	" "	27	" " "
68	Carcinoma of the skin	26	" " "
69	" "	26	" " "
70	" "	25	" " "
71	" "	24	" " "
72	Carcinoma of the lip	23	" " "
73	Carcinoma of the larynx	22	" " "
74	Carcinoma of the ovary	20	" " "
75	Carcinoma of the lip	20	" " "
76	Pigmented melanoma of the skin	17	Tumour present
	Mean	24 ± 4.3	

*For definition of units, see text.

which cannot be sufficiently evaluated by routine examination and diagnostic procedures.

If this view is valid, it might be possible to detect and differentiate between two ribonucleases in the blood serum of cancer patients: one which originates from neoplastic tissue and one which is found normally in blood serum.[6]

At present we have no knowledge that these two kinds of enzyme exist in human blood serum. An alternative explanation for the high serum RNase level in malignant disease might be that the enzyme originates from the blood cells themselves. The high level of ribonuclease might then be evidence of one of the organism's defence mechanisms against injury. The injured tissue, therefore, would not be the source of the RNase in the blood stream, but rather triggers a mechanism which releases the enzyme into the blood serum from the blood cells themselves.

SUMMARY

The serum RNase level in 60% of patients with malignant disease was significantly higher than that found in normal subjects. In a group of patients with cancer who were considered to have been successfully treated and with no signs of residual disease, the serum RNase level was increased in 30%. The possible causes of this high serum ribonuclease level in such patients are discussed.

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Testicular Adenocarcinoma with Clear Cells Occurring in Infancy: A Distinctive Tumour

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DURING the past 15 years we have studied 13 examples of a distinctive glandular, papillary and clear-celled adenocarcinoma in the testis of infants. We pointed out previously[1, 2] that this testicular neoplasm was apparently peculiar to infancy, a fact that had escaped attention in the few individual cases published prior to our reports.[3, 4] In 1960, Teoh, Steward and Willis[5] described 15 cases of this neoplasm and agreed with our view that this kind of tumour is morphologically distinctive and characteristic of infancy. We record here our complete series of 13 cases, including six cases not previously reported.

PATHOLOGY

Grossly these tumours expanded the testicle to a greater or lesser degree, without breaking through the tunica albuginea. The smallest tumour in our series measured 2.5 cm. and the largest 8.0 cm. in diameter. The consistency was usually firm. The cut surfaces were white, pale-yellow or brownish, sometimes solid, sometimes honeycombed by small cystic spaces (Fig. 1). Often the cut surfaces were covered with a layer of sticky fluid.

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Microscopically the most characteristic feature was heavily vacuolated tumour cells, arranged in loosely woven masses, irregular tubular spaces and small cystic cavities (Figs. 2-5, and Fig. 6); the cavities sometimes had delicate papillary formations. Special stains indicated that these vacuoles often contained glycogen and sometimes mucin, usually in low concentration. Three tumours, in which frozen sections were made and fat stains done, showed many of the cells laden with sudanophilic droplets. Also present were non-vacuolated cuboidal cells forming tubular structures and lining cystic spaces (Fig. 7). The stroma, which varied in quantity, consisted of loose fibrillary connective tissue, sometimes mucoid or edematous in character, and less frequently contained more dense collagenous septa.

CLINICAL FEATURES

Testicular enlargement during the first two years of life was the presenting symptom in every case. In five children the enlargement was first attributed to hydrocele and in one child to trauma. Orchiectomy followed recognition of the scrotal mass at periods varying from one month to six months and constituted the only treatment in eight cases. Five of the patients received postoperative irradiation, one of these for palliation of metastatic lesions (see Table I).